Influence of age on the physiological serum alpha-fetoprotein levels in man and rat

R. MASSEYEFF, J. GILLI, C. BONET and H. ZRIHEN

Faculté de Médecine et Centre Antoine Lacassagne, Nice, France

SUMMARY

In the early days of the discovery of alpha-fetoprotein (AFP), it was demonstrated by conventional immunodiffusion techniques that nearly all new born sera from animals as well as from human babies contain this protein[1]. Quantitative studies have shown that the levels are in the range of 60,000–130,000 ng/ml in human new born[2] and 5, 10^6 ng/ml in the rat[3]. Conversely, the same techniques were unable to detect AFP in adult sera. However, by the highly sensitive radioimmunoassay (RIA) method, several workers were able to demonstrate that adult sera do contain a very small amount of AFP. Table 1 gives the normal levels as determined by different workers.

Very little is known about the transition between the high levels observed at birth and the low adult levels. The aim of this work was to study the physiological serum AFP concentration in man and in the rat throughout life.

MATERIALS AND METHODS

Sera

201 samples of blood were drawn from clinically healthy children, from a few weeks to 15 years old. No follow up study was made. 192 sera were obtained from healthy blood donors aged 20–60. 16 sera came from people between 60 to 98. Males and females were in nearly equal number in all these samples. Furthermore, samples of blood were drawn from normal Sprague-Dawley rats ranging from a few days to two years. Sera were separated by centrifugation and stocked frozen at −30°C until assayed.

Radioimmunoassay (RIA)

AFP was determined by RIA method fully described elsewhere[4], the main features of which are given below: human AFP was isolated from the serum of a patient with primary liver carcinoma using the method of

Nishi[5]. Rat AFP was obtained from fetal serum by the same method.

Antisera were raised in rabbits in the laboratory using purified AFP. Althogh apparently specific when using conventional immunodiffusion methods, they were further absorbed using an immunosorbent made of normal serum.

RIA was done by the double antibody method of Morgan and Lazarow[6] with a few modifications, using ^{125}I labeled AFP. A specific feature of this assay is the use of chicken serum and chicken albumin as diluent, as a substitute for bovine albumin commonly used in RIA methodology. This latter reagent was avoided in case that some commercial batches may contain a cross reactive bovine AFP. The anti-AFP rabbit antiserum was diluted 1 : 40,000 for human AFP assay and 1 : 125,000 for rat AFP. At this concentration 20% of the labeled antigen is bound. A diluted sheep anti-rabbit IgG was used to precipitate the anti-AFP antibody. The radioactivity of bound AFP present in the precipitate was counted in a Packard gamma scintillation spectorometer. Pure AFP from a different batch was used for calibration.

RESULTS AND DISCUSSION

This RIA method allowed the detection of AFP with a sensitivity threshold of 0.1 ng/ml of serum for human AFP and 1 ng/ml for rat AFP. The precision of this RIA varies with AFP concentration. The coefficient of variation calculated in repeated assays of the same sample lies between 5 and 10% for AFP concentrations in the range of 0.1–5 ng/ml. Specificity of the assay was confirmed by the following experiments :

a) Further absorption of the antiserum with normal plasma did not change the observed values.

b) Parallel curves were obtained on semi-log plots, when several dilutions of samples were compared with dilutions of pure AFP standard solutions.

c) Assays of the different electrophoretic fractions of a serum containing AFP showed inhibitory activity only in the alpha-1 area.

d) Absorption of a serum containing AFP with an anti-AFP antibody immunosorbent completely removed the inhibitory activity.

From our results the following conclusions are reached :

1) As shown by Fig. 1 there is a steep decrease of AFP levels during the first year of human life. Normal adult levels are reached by the end of the second year and often six months earlier.

2) The values found for the first year are highly scattered. The highest levels were not related to prematurity. Once the adult level is

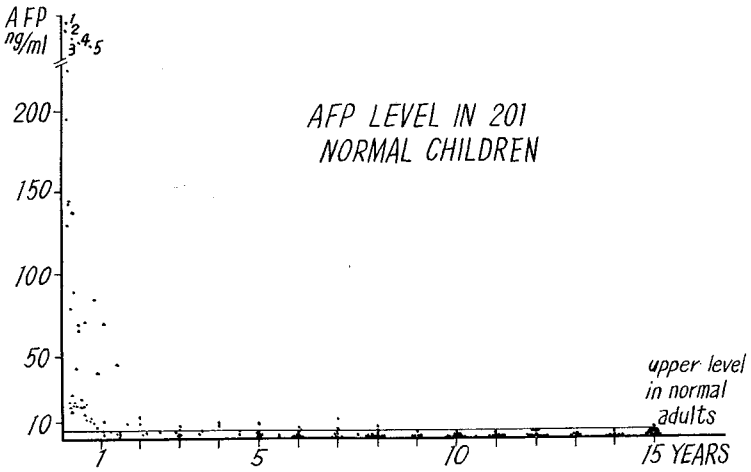

Fig. 1. AFP serum level in 201 healthy children. Results numbered 1–5 are above the scale limit.

1: more than 10,000 ng/ml
2: 2,075 ng/ml
3: 330 ng/ml
4: 325 ng/ml
5: 312 ng/ml

reached, it remains fairly stable.

3) Normal adult levels are stable between 20 to 60 years. We did not observe any significant change during the period as observed by Chayvialle and Gangulis[7]. The average concentration was 2.6 ng/ml with a standard deviation of 1.6 ng/ml.

4) AFP level is not influenced by sex nor by puberty. This apparent lack of correlation with sex hormonal conditions is of interest, since two groups have recently described the strong binding power of AFP towards oestrone and oestradiol[8,9].

5) The average level in a group of 15 normal people aged 60 to 98 was 2.0 ng/ml with a standard deviation of 1.1 ng/ml. When considering the small group of the 10 people aged over 74, the average concentration falls to 1.4 ng/ml with a standard deviation of 0.5 ng/ml. The difference with adult level is significant at $p \leqslant 0.05$.

6) Rat AFP behaves quite differently. Fig. 2 shows the variations observed between the fourth and the tenth week of life. At this time the concentration of AFP is in the range of 25 ng/ml. The sera of older rats were also assayed. However, the ages of these latter rats were not known with precision. When the results were plotted according to rat weight, a striking decrease of AFP level was found as shown in Fig. 3. It seems

highly likely that weight is strongly correlated with age in these captive rats and that decrease of AFP levels reflects the influence of age rather than weight. The heaviest rats (more than 400 g) were old rats (about 2 years old). The average AFP level in this group was 9.9 ng/ml. No influence of sex nor of puberty was found. Furthermore, female rats were castrated before and after puberty and their levels did not differ significantly from control rats.

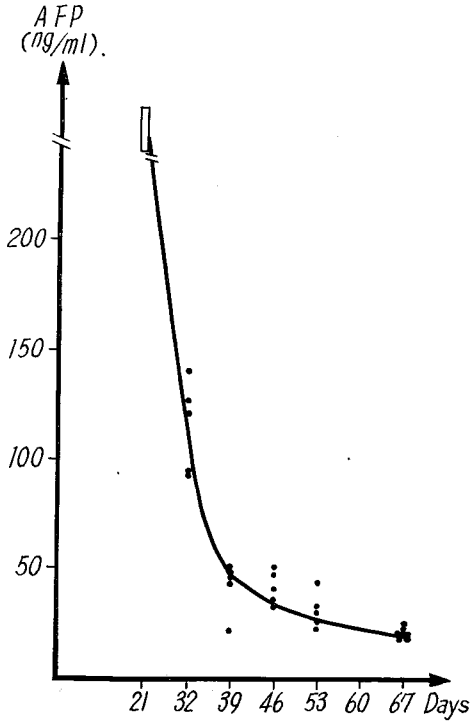

Fig. 2. AFP serum level in normal rats from 4 to 10 weeks old

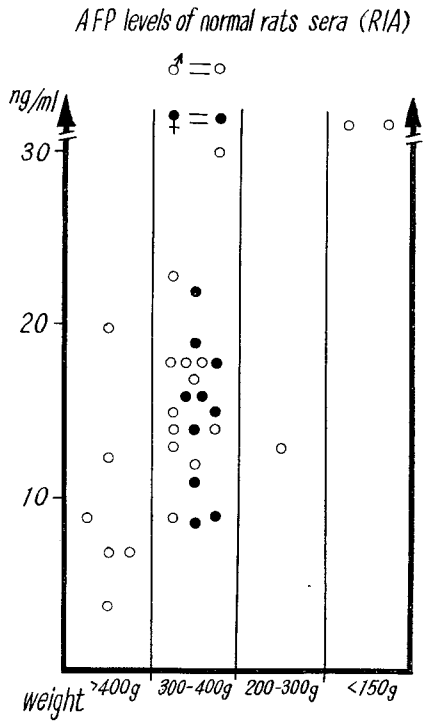

Fig. 3. AFP serum levels in normal rats in relation to weight. The heaviest rats (more than 400 gr.) were old rats (about 2 years old)

The levels of serum AFP found in normal adults are in the same range than those found by other workers as shown in Table 1. The disagreement between the different values are minor compared to the strong discrepancies found in the literature for the normal values of some other serum trace components, such as carcino-embryonic antigen. However, we, as other workers, still do not consider the values found in normal subjects as quantitatively accurate, since they are close to the sensitivity limit of the assay.

Table 1. *Alpha-fetoprotein serum level in normal adult*

	Average normal adult AFPserum level (ng/ml)	Standard deviation or range	Authors
Man	10	1-20	Purves *et al.*
	7.6	1-16.5	Ruoslahti and Seppälä
	2.3	±1.2 ng/ml	Chayvialle and Ganguli
	2.6	±1.6 ng/ml	This work
Rat	26	±12	Sell and Gord
	16.6	± 5.4	This work

The observed discrepancies may result from technical differences. The most immediate explanation is that the pure AFP preparations used for calibration were not the same. They may differ biochemically. Several workers have described molecular variants of AFP. Purves insisted on the different antigenic activity of these variants[10]. Even with the same molecular form, the preparation used for calibration may still be impure, or it may be partly denatured by the processing. The effect would be the same in both cases, resulting in overestimated levels. We do not have evidence for any of these possibilities in our assay. Obviously, an internationally accepted reference preparation is needed. Future results should be expressed not only in terms of ng/ml, but in fractions of this reference, allowing for comparisons of assays made in different laboratories.

This study confirms that the post natal reduction of AFP biosynthesis is not complete and shows that it is a gradual process both in the rat and in man. If synthesis in man were reduced to the adult rate soon after birth, the observed concentration of AFP would reflect the spontaneous catabolism of this protein. The catabolic period of this protein is not known with precision although times of 2.5 and 6.2 days have been reported. Taking extreme values of 2.5 and 6.0 days for the catabolic period, and assuming a birth level of 80,000 ng/ml, it would take 15 periods to reach the adult level of 2.5 ng/ml, i.e. 38 and 90 days respectively. But as shown in Fig. 1, the adult level is not reached until much later.

In the rat, this reduction in the biosynthesis of AFP is more gradual but does not reach a plateau as in man and continues to decline throughout life.

From the literature and from preliminary experiments it seems that AFP production is not as strictly repressed in childhood as in adult life. The pattern of AFP biosynthesis at varying age periods can be modified,

in adults as in children, particularly by the supervention of liver diseases such as hepatitis and carcinoma. In children, moreover, diseases other than hepatic diseases, such as non specific infections and even trauma, can stimulate an increase in the production of AFP yielding a concentration of 50–200 ng/ml. Investigations are in progress to elucidate the nature of these reactions.

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